# Responses to Hedgehog

## Protein kinase A activity is required for signalling by the extracellular molecule Hedgehog in developing *Drosophila* imaginal discs, but does the kinase actually respond to the Hedgehog signal?

Spatially appropriate developmental decisions frequently rely on the exchange of information between cells. Recently, genetic studies of *Drosophila* imaginal disc development have linked the cAMP-dependent protein kinase A (PKA), a component of a well-established signal transduction pathway, to the extracellular signalling molecule, Hedgehog [1–5]. Hedgehog family members are key inducers of major patterning events in vertebrates and invertebrates, and hence provide an important focus for a comparative study of cell–cell communication in distantly related organisms. The objectives of such studies are to ascertain the biochemical responses elicited by Hedgehog, and to understand the spatial and temporal specificity of Hedgehog signals and their information content. Although few definitive answers are available, several interesting questions have been raised by recent results. Is there a single Hedgehog signalling protein, a single Hedgehog receptor or a single Hedgehog signal transduction pathway? What are the biochemical connections between the different molecules that have been genetically implicated in Hedgehog signal reception? What determines the range of a Hedgehog signal? And is Hedgehog signalling dose-dependent?

Selective expression of a single *Drosophila hedgehog* (*hh*) gene serves at least three different purposes during development: the maintenance of expression of the *wingless* (*wg*) signalling molecule in cells adjacent to *hh*-expressing cells early in embryogenesis [6]; the dosage-dependent long-range determination of dorsal epidermal cell fates later in embryogenesis [7]; and a long-range effect on the growth and patterning of imaginal discs, the progenitors of adult structures such as legs, wings and eyes. Studies *in vitro* have shown that the primary *hh* translation product yields a transmembrane protein, Hedgehog, which can be processed by cleavage after a signal sequence and by autoproteolysis to yield at least three major extracellular forms, all of which can be found in extracts from embryos or imaginal discs [8]. This suggests that the range of Hedgehog signalling might be controlled by proteolysis and that separate domains of the Hedgehog protein might have distinct signalling activities. But there are other ways to modulate the apparent range of a Hedgehog signal, including the local induction of secondary signalling molecules.

The requirements for Hedgehog receptors in different cell populations would provide a direct measure of the effective signalling range of Hedgehog ligands. One candidate receptor is the integral membrane protein Patched, which is expressed in Hedgehog-responsive cells of imaginal discs and early embryos; mutations in the *patched* (*ptc*) gene yield phenotypes that can also be produced by ectopic *hh* expression. As ectopic expression of *hh* produces similar effects to inactivation of *ptc*, Patched would have to be an unusual receptor, with its activity inhibited, rather than activated, by binding ligand (Hedgehog). There are also reasons to believe that a receptor other than Patched must exist. The ventral cuticle of *ptc* and *ptc hh* double-mutant embryos are not identical, as would be expected if Patched is the only Hedgehog receptor. Also, *ptc* is not detectably expressed in some Hedgehog-responsive cells of the dorsal epidermis. Interestingly, ectopic expression of zebrafish and *Drosophila hh* homologs in *Drosophila* embryos elicits similar expansions of the domain of *wg* expression; as the homologs can achieve the same effects, there is likely to be conservation of the pertinent Hedgehog receptor.

Hedgehog plays a major role in patterning imaginal discs. The imaginal discs are specified during embryogenesis, but grow and differentiate as a folded epithelial sac during larval and pupal stages. In leg and wing imaginal discs, the fate of a cell depends upon its position within the two-dimensional epithelium. The early localized expression of the *engrailed* (*en*) gene in posterior disc cells and of *wg* in ventral disc cells is essential for establishing later sources of positional information. The heritable expression of *en* contributes to the establishment of a compartment boundary at which *hh*-expressing posterior cells are confronted by *hh*-responsive anterior cells (Fig. 1). Inactivation of *hh* during larval stages arrests growth in appendage discs, whereas ectopic *hh* expression in the anterior compartment promotes aberrant growth and induces spatially inappropriate cell fates [9,10]. In the eye disc, the position of cells affects only the time at which differentiation begins, and not the final cell fate. Differentiation is initiated at the site of a depression in the epithelium, known as the morphogenetic furrow, which moves from posterior to anterior. *Hh* is expressed posterior to the moving furrow and is required for furrow movement. Conversely, ectopic *hh* expression can initiate an ectopic furrow that propagates away from the source of *hh* [11]. It was recently found in *Drosophila* imaginal discs that the production of clones of cells with reduced PKA activity induces adult morphologies that resemble those due to local ectopic *hh* expression.

The influence of localized *hh* expression on growth and patterning of entire imaginal discs is thought to result

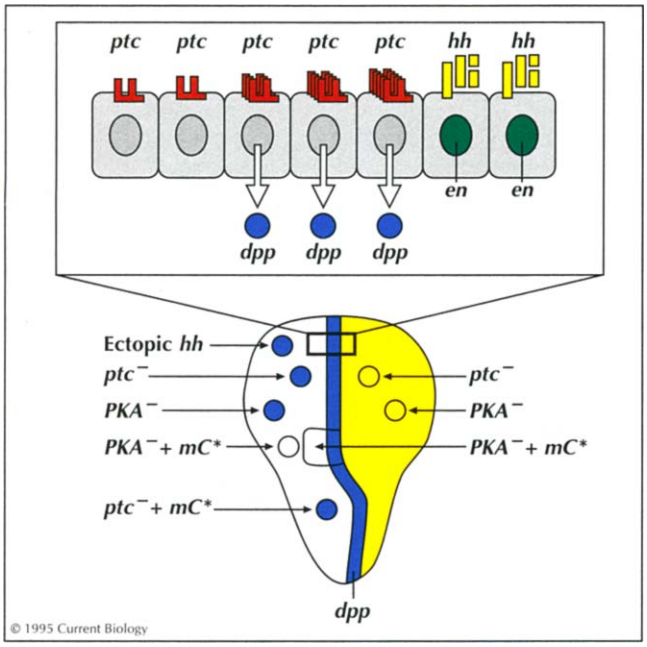



**Fig. 1.** *Hedgehog* (*hh*) induces the expression of *dpp* in a strip of about eight anterior cells (represented by three cells in the diagram) at the anteroposterior border of a third instar wing imaginal disc, despite the repressive effects of *patched* (*ptc*) and *PKA*. Clones expressing *hh* or mutant for *ptc* or *PKA* induce ectopic *dpp* expression if they are made up of anterior, but not posterior, compartment cells. Activated mouse PKA (mC*) can substitute for *Drosophila* PKA, but not for Patched, and does not abrogate Hedgehog signalling at the anteroposterior border. Expression of *dpp* in *PKA*-mutant clones is essential for the reorganization of cell fates and for growth; it may affect distant cells directly or through further inductive interactions.

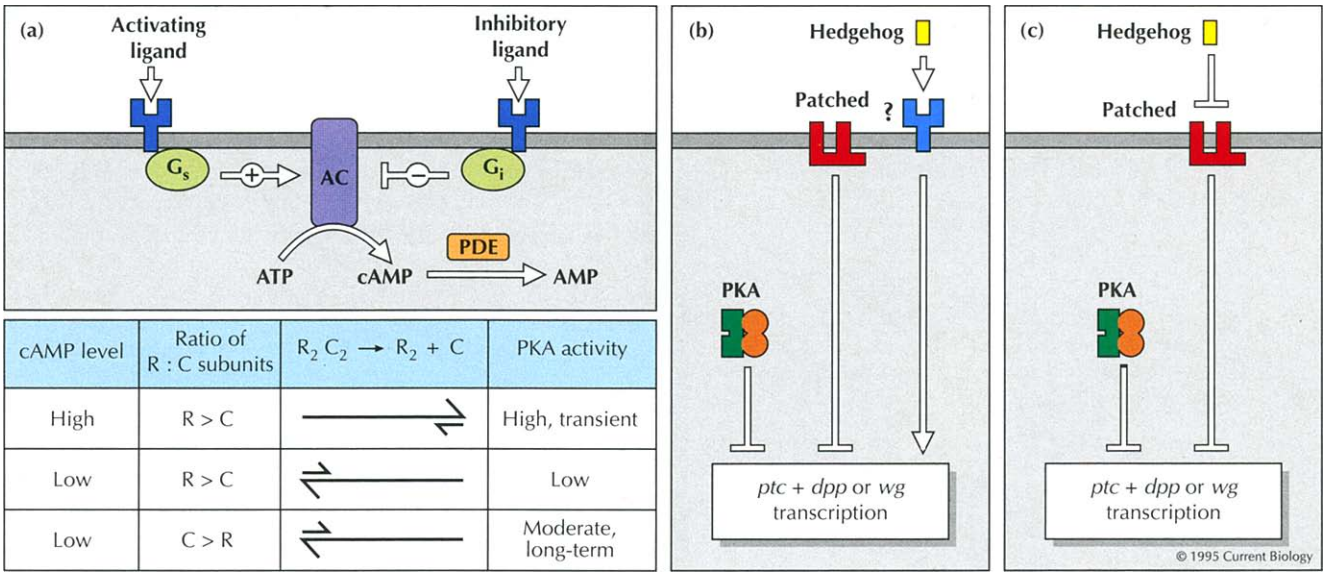
from the intermediate activation of secondary signalling molecules, such as Wingless in ventral leg discs and the TGF-β/BMP homolog, Decapentaplegic, in wing, eye and dorsal leg discs [9,10]. Several observations support this proposal. The transcription of *decapentaplegic* (*dpp*) and *wg* in a strip of cells immediately anterior to the domain of *hh* expression normally depends upon *hh* activity. Conversely, transcription of *dpp* and *wg* can be induced at ectopic locations by ectopic *hh* expression, or within clones mutant for *PKA* or *ptc* (Fig. 1). Crucially, *PKA*-mutant clones induce pattern alterations and overgrowth of wings, whereas *PKA dpp* double-mutant clones do not. This suggests that *dpp* can account for the long-range effects of *hh* (acting presumably through *PKA* and *ptc*) in wing discs. Interestingly, extensive changes in growth and cell fate were nevertheless induced in *PKA dpp wg* triple mutants in ventral regions of the legs, suggesting that there is an additional unidentified secondary signal in leg discs.

The transcriptional induction of *dpp* and *wg* is dependent on *hh* in normal discs but may be elicited by *ptc*-mutant clones or by *PKA*-mutant clones without the induction of *hh* expression [1–5] and independently of *hh* activity [3]. Does this mean that PKA is 'downstream' of Hedgehog, and that Hedgehog signal transduction involves the lowering of PKA activity? Furthermore, might the transmembrane protein Patched act to stimulate PKA? These ideas were tested by using a mutant mouse catalytic subunit of PKA, mC*, which has minimal affinity for the regulatory subunit of the kinase and should therefore be active even in the absence of cAMP. First, it was found that mC* could substitute for the major *Drosophila* catalytic subunit of PKA but not for Patched, implying that Patched does not simply regulate cAMP or the levels of PKA subunits [1,3]. Also, overexpression of *ptc* did not alter PKA activity in wing imaginal disc extracts [3]. Thus, it is very unlikely that Hedgehog modulates PKA activity via Patched. Second, clones of cells expressing mC* in place of the major *Drosophila* PKA catalytic subunit did not disturb the selective expression of *dpp* close to the anteroposterior compartment border [1], implying that Hedgehog can (at least) maintain *dpp* transcription without altering PKA activity (Fig. 1). Whether the initiation of *dpp* expression at the anteroposterior border requires modulation of PKA activity has not been tested.

How do the antagonistic effects of PKA and Patched contribute to the properties of Hedgehog signalling in imaginal discs? The basal *hh*-independent expression of *ptc* throughout the anterior compartment might serve to set a threshold for the level of *hh* required to elicit a response, thereby determining the range of the Hedgehog signal. Furthermore, the elevated *hh*-dependent transcription of *ptc* near the anteroposterior border might produce a fairly uniform differential between the opposing activities of *hh* and *ptc*, despite wide variations in the extracellular Hedgehog concentration. This would allow quantitatively similar levels of transcription of genes such as *dpp* over several cell diameters.

It is possible that arguments similar to these for Patched may apply to PKA, but at present nothing is known about the expression levels of PKA subunits in different regions of the disc. Although it would be ironic if PKA, a classical signal transducing agent, served an entirely passive role in hedgehog signal transduction (Fig. 2b,c), there is ample precedent for exploiting the basal activity of PKA in signalling pathways. In *Saccharomyces cerevisiae*, complete loss of PKA, or indeed hyperactive PKA, has severe phenotypic consequences, whereas mutations in adenylyl cyclase are without effect as long as low basal PKA activity is maintained. The essential PKA activity in anterior disc cells may result from the natural equilibrium between tetrameric (inactive) and monomeric (active) PKA at ambient cAMP levels, maintained elevation of cAMP levels, or a stable state of PKA activation due to an excess of catalytic subunit over regulatory subunit (Fig. 2a). The latter mechanism is used in rat hepatoma cells to maintain transcription of a subset of tissue-specific genes that are 'cAMP-responsive' [12]. Furthermore, it has been demonstrated in *Aplysia* neurons that prolonged activation of PKA due to a reduction in regulatory subunit levels can be induced by transient pulses of cAMP [13].

The ability to convert a transient signal into a stable response may make PKA a particularly appropriate




| cAMP level | Ratio of R : C subunits | $R_2C_2 \rightarrow R_2 + C$ | PKA activity |
|---|---|---|---|
| High | R > C | ⇌ | High, transient |
| Low | R > C | ⇌ | Low |
| Low | C > R | ⇌ | Moderate, long-term |

**Fig. 2. (a)** PKA can respond to both activating and inhibitory ligands that bind to serpentine receptors and modulate the activity of adenylyl cyclase (AC) via G proteins ($G_s$, $G_i$) to regulate the synthesis of cAMP. A number of other signals can also modulate the activity of $Ca^{2+}$-dependent adenylyl cyclases by altering intracellular $Ca^{2+}$ concentration. cAMP binds to the regulatory subunit (R) in the inactive tetrameric PKA holoenzyme, causing release of active monomeric catalytic subunit (C). Biochemical and genetic results currently favor the view that PKA activity is neither stimulated by Patched nor inhibited by Hedgehog, and do not distinguish whether Patched is a receptor for Hedgehog **(c)** or is not **(b)**. The proposed constitutive PKA activity in anterior cells that represses *dpp* transcription could be due to (1) elevated cAMP levels, (2) the basal activity of PKA at ambient cAMP concentrations, or (3) a specific signal earlier in development that led to an excess of catalytic subunits over regulatory subunits.

mediator for cellular interactions during development. Perhaps the best-documented case of long-term PKA signalling in development comes from analysis of signals that control the fate of progeny of the median neuroblast (MNB) in the embryonic grasshopper central nervous system [14]. The earliest and latest progeny of the MNB become neurons, but in the interim MNB progeny become glial cells. The second developmental transition can be delayed by PKA inhibitors or accelerated by PKA activators and is associated with the translocation of free catalytic subunit to the nucleus, where it remains for at least thirty hours, indicative of long-term activation. Perhaps there is a similar activation of PKA early in the development of *Drosophila* imaginal discs that is subsequently maintained in order to repress *hh*-responsive genes.

Future studies will clarify how PKA contributes to Hedgehog signalling and whether its involvement extends to vertebrates. It is already clear, however, from the studies described above and the recently discovered role of PKA in localizing RNAs along the anteroposterior axis during *Drosophila* oogenesis [15] that PKA, a classical multifunctional regulator of physiology, has been incorporated in a variety of different roles into the developmental programs of metazoa.

## References


1. Jiang J, Struhl G: **Protein kinase A and hedgehog signalling in *Drosophila* limb development.** *Cell* 1995, **80**:563–572.
2. Lepage T, Cohen SM, Diaz-Benjumea FJ, Parkhurst SM: **Signal transduction by cAMP-dependent protein kinase A in *Drosophila* limb patterning.** *Nature* 1995, **373**:711–715.
3. Li W, Ohlmeyer JO, Lane ME, Kalderon D: **Function of protein kinase A in hedgehog signal transduction and *Drosophila* imaginal disc development.** *Cell* 1995, **80**:553–562.
4. Pan D, Rubin GM: **cAMP-dependent protein kinase and hedgehog act antagonistically in regulating decapentaplegic transcription in *Drosophila* imaginal discs.** *Cell* 1995, **80**:543–552.
5. Strutt DI, Wiersdorff V, Mlodzik M: **Regulation of furrow progression in the *Drosophila* eye by cAMP-dependent protein kinase A.** *Nature* 1995, **373**:705–709.
6. Ingham PW: **Localized *hedgehog* activity controls spatial limits of *wingless* transcription in the *Drosophila* embryo.** *Nature* 1993, **366**: 560–562.
7. Heemskerk J, DiNardo S: ***Drosophila* hedgehog acts as a morphogen in cellular patterning.** *Cell* 1994, **76**:449–460.
8. Lee JJ, Ekker SC, von Kessler DP, Porter JA, Sun BI, Beachy PA: **Autoproteolysis in hedgehog protein biogenesis.** *Science* 1994, **266**: 1528–1537.
9. Tabata T, Kornberg TB: **Hedgehog is a signaling protein with a key role in patterning *Drosophila* imaginal discs.** *Cell* 1994, **76**:89–102.
10. Basler K, Struhl G: **Compartment boundaries and the control of *Drosophila* limb pattern by hedgehog protein.** *Nature* 1994, **368**: 208–214.
11. Heberlein U, Singh CM, Luk AY, Donohoe TJ: **Growth and differentiation in the *Drosophila* eye coordinated by hedgehog.** *Nature* 1995, **373**:709–711.
12. Boshart M, Nitsch D, Schutz G: **Extinction of gene expression in somatic cell hybrids- a reflection of important regulatory mechanisms?** *Trends Genet* 1993, **9**:240–245.
13. Bergold PJ, Beushausen SA, Sacktor TC, Cheley S, Bayley H, Schwartz JH: **A regulatory subunit of the cAMP-dependent protein kinase down-regulated in *Aplysia* sensory neurons during long-term sensitization.** *Neuron* 1992, **8**:387–397.
14. Condron BG, Zinn K: **Activation of cAMP-dependent protein kinase triggers a glial-to-neural cell-fate switch in an insect neuroblast lineage.** *Curr Biol* 1995, **5**:51–61.
15. Lane ME, Kalderon D: **RNA localization along the anteroposterior axis of the *Drosophila* oocyte requires PKA-mediated signal transduction to direct normal microtubule organization.** *Genes Dev* 1994, **8**:2986–2995.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